

ORIGINAL ARTICLE

In vitro–*in vivo* sequence studies as a method of selecting the most efficacious alcohol-based solution for hygienic hand disinfection

R. Herruzo, M. J. Vizcaino and I. Herruzo

Department of Preventive Medicine, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain

Abstract

The use of alcohol-based hand rubs serves to reduce hospital-acquired infections. Many products of this type are now on offer and it is essential to know how to rank their efficacy. A sequence of tests is proposed here to compare any given new alcohol-based solution against the reference solution (60% 2-isopropyl-alcohol) with 30 s of contact time: (i) *in vitro* (with pig skin as carrier) testing of >30 species of microorganism; (ii) *in vitro* assessment of residual efficacy (after 30 min of drying); (iii) *in vivo* study of transient microbiota (modification of the EN 1500 standard procedure) using four ATCC strains; (iv) *in vivo* study of resident hand microbiota. After performing the *in vitro* evaluation of seven alcohol-based hand rubs, the two most efficacious (chlorhexidine-quac-alcohol and mecetronium-alcohol) were chosen and studied, comparatively with the reference solution (60% isopropyl alcohol), *in vitro* (for chemical sustainability on the skin) and *in vivo* (against transient and resident microbiota). Chlorhexidine-quac-alcohol proved to be significantly superior to mecetronium-alcohol or the reference solution in all tests, except against resident microbiota for which the improvement was not statistically significant.

Keywords: Alcohol-based solutions, hands, method, resident microbiota, transitory

Original Submission: 27 October 2008; **Revised Submission:** 22 January 2009; **Accepted:** 27 January 2009

Editor: D. Raoult

Article published online: 15 July 2009

Clin Microbiol Infect 2010; **16**: 518–523

10.1111/j.1469-0691.2009.02827.x

Corresponding author and reprint requests: R. Herruzo, Departamento de Medicina, Preventiva, Facultad de Medicina, UAM c/ Arzobispo Morcillo, nº 4, 28029, Madrid, Spain
E-mail: rafael.herruzo@uam.es

Introduction

Hand washing has traditionally been a key element in preventing colonization and hospital-acquired infections caused by transient microbiota. Nevertheless, the rate of compliance with this basic rule is under 50% [1–3] for a variety of reasons: time, location of sinks, confidence in its efficacy, dermal irritation from continuous use, etc. [4–8]. Hence efforts are being made to enhance compliance by changing the attitudes of hospital healthcare workers (HCWs) to the use of alcohol-based hand rubs. These products are fast-acting bactericidal solutions that can be placed at the patient's bedside, are less skin irritating, and are more efficacious than handwashing [9,10], and, unless hands are visibly soiled [11], application of these solutions avoids the need to go to a sink to wash them.

The use of these solutions has been very effective inasmuch as they have reduced both the transmission of antibiotic-resistant microorganisms and the number of hospital-acquired infections [12–17].

At present, a wide range of alcohol-based hand rub solutions are available for hygienic hand disinfection, but it is essential to know how to rank them so as to identify those that are significantly better than the others. At present the only requirement for bringing a product to market is to meet certain standards in *in vitro* trials, such as the EN 1040 [18] or EN 1215 [19] (a test that is extremely easy to pass since the microorganisms are in suspension), or the EN 1500 [20], an *in vivo* trial, which only requires that the new product's efficacy be 'no less than that of 60% 2-isopropyl-alcohol in 1 min'. It should be possible to compare the different

Correction added on 19/03/2013 after initial online publication. A duplicate of this article was published under the DOI: 10.1111/j.1198-743X.2009.02827.x. This duplicate has now been deleted and its DOI redirected to this version of the article.

alcohol-based solutions with one another using these tests, but we also need another method. Moreover, the EN 1500 can not be used to calculate the 'real' \log_{10} reduction of the initial inoculum because the artificial contamination of volunteers' hands is done by immersing their fingers in a broth culture and letting them air dry for 5 min, and the control is made by immersing the fingertips in a sterile broth for 1 min. The result of this is that many of the microorganisms are removed from the hands and remain in this control broth to be counted after incubation. The hands are then rinsed under running tap water for 15 s (which removes more microorganisms), and it is on this 'residual colonization' of the hands that the antiseptic is applied before taking the efficacy sample. More properly, the alcohol-based solution should be applied to hands contaminated to similar extents and with similar microorganisms in both tests and controls. Furthermore, the EN 1500 test uses only one control strain, namely *Escherichia coli* ATCC, so that the results obtained may well not be applicable to other microorganisms, for example, other ATCC strains, and perhaps even less applicable to patient strains, which tend to be far more resistant to biocides than are ATCC strains [21].

Another aspect that should be ascertained is the efficacy of these solutions against the resident microbiota on HCWs' hands. If the products are intended for surgical handwashing purposes it would be necessary to assess whether any products have chemical sustainability on the skin [22–25]. This is important, because otherwise the resident microbiota can recuperate under the glove during the intervening period and may even exceed their pre-wash density [22].

Materials

Alcohol-based solutions

The following solutions were tested: (i) Daromix-solucion (Biguan-prop-et): 0.2% biguanidine, 2-propanol and ethanol (Lab Jose Collado, Barcelona, Spain); (ii) Pentabiot (Phenox-prop-et): phenoxyethanol, ethanol, 1-propanol (Lab Hydenet, Sainghin-en-Melantois, France); (iii) Sterillium (mecetronium-alcohol): 0.2% mecetronium, 1,2-propanol (Lab Bode-Chemie GmbH, Hamburg, Germany); (iv) SAM (chlorhexidine-quac-alcohol): 0.3% Chlorhexidine, 0.8% didecyl-polyoxyethyl-ammonium propionate, 1,2-propanol (Lab Inibsa, Lliça de Vall, Barcelona, Spain); (v) ADH 2000 (Et-butanodiol): ethanol, 2-butanodiol (Lab Lysoform, Berlin, Germany); (vi) NDP-derm (N-duoprop-et): N-duopropenide, ethanol (Lab Vesismín, Barcelona, Spain); (vii) Septoderm (Prop-butanodiol): propanol, butanodiol (Lab Dr Schumacher, Melsungen, Germany); (viii) control solution: 60% 2-isopropyl-alcohol.

Microorganisms and culture media

The microorganisms used in this study included: four ATCC strains (*E. coli* K12, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 18804); 32 microorganisms recently (<2 days) isolated from ICU patients including ten *Enterobacteriaceae*, ten non-fermentive bacteria (NFB), seven Gram-positive cocci and five yeasts.

Tood Hewitt broth containing Tween (80.6%), sodium bisulfite (0.5%) and sodium thiosulfate (0.5%) was used as antiseptic neutralizer. Petri dishes with mannitol salts agar (Biomedics), or Cand2 (Biomerieux), MacConkey agar (Difco) or blood agar (Biomerieux) were acquired with help from the Bode-Chemie, Inibsa and AGB laboratories, through Fundacion Universidad Autonoma de Madrid.

Methods

In vitro method to detect the efficacy of alcohol-based solutions

A method similar to one previously described [8,22,26] was adapted to shorten the experiment time to 30 s. In brief, lyophilized pig skin was cut into circles 0.5 cm in diameter, sterilized under steam flow, introduced into the culture medium with the microorganism under test, and left for 15 min. This carrier was then introduced into an alcoholic solution and after 30 s the germicidal action was stopped by addition of the neutralizer. One gram of sterile glass beads (1 mm diameter) was added to 5 mL of neutralizer and shaken at 1000 rpm for 1 min. Two supernatant samples of 0.1 mL (made to a dilution of 1/100) were taken and spread on the Tris-buffered saline agar surface. CFUs were counted after 48 h incubation at 37°C. The controls were processed in the same manner except that water was used instead of disinfectant, and samples were diluted 1/100 and 1/10 000, before being sown on the Petri dish.

In vitro method for assessing the chemical sustainability of alcohol-based solutions on the skin

Microorganism carriers similar to those in the first method were used except that, for this test, they were impregnated with 20 μ L of the alcohol-based solution that was being assessed. The carriers were allowed to dry for 30 min, after which time they were covered with 10 μ L of a microbial culture of bacteria and left to act for 1 min. The antiseptic action was interrupted by immersing the germ carriers in the neutralizing broth, and then proceeding as in the first method. The control procedure was similar in all respects, except that sterile distilled water instead of alcohol-based solution was used. The bacterial sample was then incubated for 48 h, and the \log_{10} was calculated and recorded.

In vivo study (modification of the European EN 1500 standard procedure)

The EN 1500 procedure was modified in various respects.

- 1 *Microorganisms, media and volunteers.* Not only *E. coli* ATCC but also *P. aeruginosa* ATCC, *S. aureus* ATCC and *C. albicans* ATCC were used. They were seeded in suitable culture media to facilitate the microbial count without interfering with the volunteers' resident cutaneous microbiota (MacConkey for *P. aeruginosa* and *E. coli*, Cand2 for *C. albicans*, and mannitol for *S. aureus*). In the last case it was necessary to exclude any volunteers who were *S. aureus* carriers. This required a prior study of nasal and subungual colonization in order to prevent false-positive results for *S. aureus*.
- 2 *Assessment of the biocidal effect.* Initial contamination in each volunteer was assessed, after handwashing, according to the EN 1500 standard method, with samples being taken in a similar fashion from each hand for CFU counting. This constituted the control. At this point, however, instead of rinsing and antiseptic treatment, volunteers again washed and dried their hands, which were re-contaminated and allowed to dry for a further 5 min, before application of 3 mL of antiseptic to both hands. The rest of the procedure was performed according to EN 1500, except for the fact that contamination was now similar to that used for the control. The study time was only 30 s.

In vivo study to assess the efficacy on the resident microbiota of HCWs' hands

In this case, one hand acted as the test (the dominant hand, which tends to be more colonized) and the other as the control. First, a sample was taken from the control hand, by immersing the finger tips in 10 mL of broth culture for 1 min (as per the EN 1500). The hands were then rinsed under running tap water for 1 min and dried with a towelette for a further minute. After this, 3 mL of alcohol-based solution was applied to the hands, spread as in normal hygienic hand disinfection and, after 30 s, the finger tips of the hand were pressed into broth cultures containing the neutralizers of the antiseptic action. Finally, samples of this neutralizer were taken in accordance with the EN 1500 standard procedure. The difference between the log₁₀ CFUs observed for the control and the test hands was the resulting log₁₀ reduction.

Statistical analysis

The log₁₀ reduction obtained with the alcoholic solutions was studied as follows: in the *in vitro* study with the 36 microorganisms, the MANOVA test was performed for multiple

comparisons of the log₁₀ reductions obtained with the eight alcohol-based solutions against the microorganisms of the groups of enterobacteria, NFB, Gram-positive cocci and fungi. A value of $p < 0.05$ was deemed significant. The three most efficacious solutions (and the reference alcohol) were selected for subsequent study.

The residual effect was studied using ANOVA, comparing in the same test the selected alcohol solution against the reference alcohol.

In the *in vivo* study with acquired microbiota, a T-paired test was performed, comparing isopropyl-alcohol efficacy with that of the three other products among all ATCC strains used. The mean log₁₀ reductions observed with each product against acquired or resident microbiota were compared using a t-test.

Results

In vitro tests

The efficacy of the seven initially chosen alcohol-based solutions against a broad spectrum of microorganisms (four ATCC reference strains and 32 strains recently isolated from ICU patients) is shown in Table 1. The most efficacious against these microorganisms in 30 s was chlorhexidine-quac-alcohol ($p < 0.05$), since it proved very effective at reducing the inocula of *Enterobacteriaceae*, Gram-positive cocci and NFB; the next most efficacious hand rub was mecetronium-alcohol.

The chemical sustainability on the skin of the two most efficacious solutions and of the reference alcohol are shown in Table 2. After these products had dried for 30 min (and the microorganisms then exposed to them for 1 min), the action was pronounced with chlorhexidine-quac-alcohol

TABLE 1. Mean effect (log₁₀ reduction of inoculum) of seven alcohol-based solutions on 36 microorganisms after 30 s contact time with hand rub: *in vitro* study

Mean (and SD) of log ₁₀ reduction					
Product	Entero bacteria n = 11	Gram+ cocci n = 8	NFB ^a n = 11	Fungi n = 6	Mean ± SD all microorganisms n = 36
Chlorhexidine-quac-alcohol	5.2	5.3	5	3.8	4.9 ± 0.6
Mecetronium-alcohol	4.5	3.5	4.7	2.8	4.05 ± 0.9
Phenox-prop-et	3.9	3.4	4.6	2.9	3.6 ± 0.8
Biguan-prop-et	2	2.05	1.6	1.7	1.7 ± 0.4
Et-butanodiol	2.2	2	3.5	3.6	2.5 ± 0.6
N-duoprop-et	1.8	1.7	3.1	2.5	2.2 ± 0.4
Prop-butanodiol	2.3	1.9	2.4	1.3	2.1 ± 0.3

^aNFB, non-fermentative bacteria.

TABLE 2. *In vitro* study testing of chemical sustainability on the skin of three alcohol-based solutions 30 min after application; monitoring three types of microorganisms obtained from ICU patients (contact time, 1 min)

Mean of log ₁₀ bacterial reduction ^a				
Product	<i>Escherichia coli</i> n = 4	<i>Pseudomonas aeruginosa</i> n = 4	MR- <i>Staphylococcus aureus</i> n = 4	Mean of 12 microorganisms
60% alcohol	0.15	0.1	0.7	0.3 ± 0.4
Chlorhexidine-quac-alcohol	4.1	4	3.6	3.9 ± 0.8
Mecetronium-alcohol	1.4	0.43	2.05	1.2 ± 0.5

^aLog₁₀ CFU (sample of germ carrier without alcohol solution)–log₁₀ CFU (sample of germ carrier with alcohol solution and drying during 30 min).

TABLE 3. Effect of three alcohol-based solutions on transient or resident microbiota, at 30 s: *in vivo* study

Mean and SD of log ₁₀ bacterial reduction after application of alcohol-based solutions					
Transient microbiota ^a				Resident microbiota ^b	p Log ₁₀ reduction in transient vs. resident microbiota
Product	Microorganism ^a	X ± SD	Mean 4 ATCC		
60% 2-iso-propyl alcohol	<i>E.c.</i>	4.1 ± 0.4	3.5 ± 0.9	2.7 ± 0.5	<0.05
	<i>P.a.</i>	3.3 ± 0.35			
	<i>C.a.</i>	2.9 ± 0.5			
	<i>S.a.</i>	4.1 ± 0.5			
Chlorhexidine-quac-alcohol	<i>E.c.</i>	4.4 ± 0.3	4.4 ± 0.7	3.1 ± 0.5	<0.05
	<i>P.a.</i>	4.1 ± 0.35			
	<i>C.a.</i>	4.1 ± 0.6			
	<i>S.a.</i>	5.1 ± 0.25			
Mecetronium alcohol	<i>E.c.</i>	4 ± 0.4	3.9 ± 1	2.6 ± 0.9	<0.05
	<i>P.a.</i>	4.1 ± 0.6			
	<i>C.a.</i>	3.4 ± 0.55			
	<i>S.a.</i>	3.8 ± 0.3			

E.c., *Escherichia coli*; *P.a.*, *Pseudomonas aeruginosa*; *C.a.*, *Candida albicans*; *S.a.*, *Staphylococcus aureus*.
^aTwenty volunteers successively contaminated with ATCC strains.
^bTwenty-five volunteers with their natural microbiota, studied over 3 weeks.

(capable of destroying a microbial inoculum even half an hour after their application) but small-to-moderate with mecetronium-alcohol and almost nil with 60% 2-propanol.

***In vivo* tests**

Only the two antiseptics showing greatest efficacy in the previous test and the 60% 2-isopropyl-alcohol reference solution were used to study the microbiocidal effect of these products over a 30-s period when tested against four ATCC microorganisms on 20 volunteers (Table 3). We observed that against *E. coli*, the effects of the three alcohol-based hand rub solutions were the same. The two commercial products proved significantly ($p < 0.05$) more efficacious against *P. aeruginosa* than 60% alcohol; mecetronium-alcohol was more efficacious against *C. albicans* and *S. aureus* than 60% alcohol, but it was inferior ($p < 0.05$) to chlorhexidine-quac-alcohol.

Comparison of the average effect of the above alcohol-based solutions against the four ATCC microorganisms used in both *in vitro* and *in vivo* trials (Tables 1 and 3) showed that the effect obtained in both tests was very similar (*in vitro* tests have been good predictors for what happens in *in vivo* tests with acquired microbiota).

In the *in vivo* test, the three alcohol-based solutions displayed non-significant differences (2.6–3.1 log₁₀ reductions, $p > 0.1$) in efficacy against the resident microbiota of the study subjects (Table 3), although the hand rubs that generated a greater log₁₀ reduction in microorganisms were those that had also proved more efficacious in the previous experiments.

When the effects on resident and transient microbiota were compared, the effect of the alcohol-based solutions was more pronounced (with $p < 0.05$) on transient than on resident microbiota in all cases (Table 3).

Discussion

The methods used allow the log₁₀ reduction of microorganisms to be calculated in a similar way, and thus provide accurate data on comparative efficacy. The EN 1500 test can be used to compare the efficacy of an alcohol-based solution (at 30 s or, alternatively, at 1 min) with that of a reference solution (60% 2-isopropanol at 1 min), but only against *E. coli*. This test does not reveal any variations in the effects against different microorganisms. In fact, Table 3 shows that the

effect against *E. coli* is not reflected in the results for the other microorganisms, *Candida* being more and the *S. aureus* less resistant.

Both the EN 1500 standard method and the modified version proposed here rely exclusively on ATCC microorganisms, as is logical when using volunteers. However, to extrapolate the efficacy obtained with these strains to that shown towards microorganisms that colonize or infect patients is risky, since the latter tend to be more resistant to biocides than are the ATCC strains (due both to the ageing effect and to acquisition of antibiotic resistances, which are sometimes associated with greater resistance to biocides) [21]. Accordingly, microorganisms recently isolated from patients should always be studied. If this is done, the results can be extrapolated to clinical practice. However, to ensure that no healthy volunteers are exposed, the study can be performed *in vitro*, using a test that discerns log₁₀ reductions equal to or only slightly less than those obtained in the *in vivo* experiment, so as to prevent erroneous overestimation of product efficacy.

As has been shown, resident microbiota are far more resistant to being eliminated than are transient microbiota. Consequently, the effect obtained on the latter cannot be extrapolated to estimate the effect that would be obtained on the former as the difference is greater than a log₁₀ value of 1 in all four of the studied cases. Therefore it is also necessary for efficacy against resident microbiota that they be tested against all alcohol-based hand rub solutions. Moreover, the main limitation of this study could be the extrapolation of results from resident microbiota obtained in this study to the effect on other people, because the resident microbiota can differ according to the volunteer's age, occupation in the hospital, frequency of handwashing etc.

In conclusion, the logical order of performing the three tests on which to base recommendations to include a new alcohol-based solution for hospital care-givers, would be as follows: (i) performance of an *in vitro* study, with a broad spectrum of microorganisms, thereby allowing the best disinfectant to be chosen for the ensuing *in vivo* tests and assessment of the product's chemical sustainability on the skin; (ii) modification of the EN 1500 standard procedure to increase the number of ATCC strains that allows comparison with the control sample in such a way as to ensure proper calculation of the log₁₀ reduction obtained with each product; (iii) performance of an efficacy study of resident microbiota on the hands of volunteers. Using the method outlined in this paper, with the exception of tests against resident microbiota, where the increased efficacy did not reach significance, chlorhexidine-quac-alcohol proved to be significantly supe-

rior to mecetronium-alcohol or the control solution in all the tests.

Acknowledgements

The authors would like to thank our laboratory technician, M. del Carmen Fernández-Uriarte.

Transparency Declaration

The authors declare that they have no conflicts of interest.

References

1. Pittet D. Compliance with hand disinfection and its impact on hospital acquired infections. *J Hosp Infect* 2001; 48 (suppl A): 40–46.
2. Thompson BL, Dwyer DM, Ussery XT et al. Handwashing and glove use in a long-term care facility. *Infect Control Hosp Epidemiol* 1997; 18: 97–103.
3. Pittet D. Hand hygiene: improve standards and practice for hospital care. *Curr Opin Infect Dis* 2003; 16: 327–335.
4. Rotter ML. Arguments for alcoholic hand disinfection. *J Hosp Infect* 2001; 48 (suppl A): 4–8.
5. Rotter ML, Koller W, Neumann R. The influence of cosmetic additives on the acceptability of alcohol-based hand disinfectants. *J Hosp Infect* 1991; 18: 57–63.
6. Voss A, Widmer AF. No time for handwashing!? Handwashing vs. alcoholic rub: can we afford 100% compliance? *Infect Control Hosp Epidemiol* 1997; 18: 205–208.
7. Larson E. Skin hygiene and infection prevention: more of the same or different approaches? *Clin Infect Dis* 1999; 29: 1287–1294.
8. Herruzo-Cabrera R, Garcia-Caballero J, Fernandez-Aceñero MJ. A new alcohol-based solution (N-duopropenide) for hygienic (or routine) hand disinfection is more useful than classic handwashing: *in vitro* and *in vivo* studies in burn and other intensive care units. *Burns* 2001; 27: 747–752.
9. Kampf G, Ostermayer C. Efficacy of alcohol-based gels, compared with simple hand wash and hygienic hand disinfection. *J Hosp Infect* 2004; 56 (suppl 2): 13–15.
10. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrub and rub. *Clin Microbiol Rev* 2004; 17: 863–893.
11. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings. Recommendations of the Healthcare Control Practices Advisory Committee and HICPAC/SHEA/APIC/IDSA. Hand Hygiene Task Force. *MMWR Morb Mortal Wkly Rep* 2002; 51: 1–44.
12. Boyce JM. MRSA patients: proven method to treat colonization and infection. *J Hosp Infect* 2001; 48 (suppl A): 9–14.
13. Pittet D, Hugonnet S, Harbarth S et al. Effectiveness of a hospital-wide program aimed at improving compliance with hand hygiene. *Lancet* 2000; 356: 1307–1312.
14. Goldmann D, Larson E. Hand-washing and nosocomial infections. *N Engl J Med* 1992; 327: 120–122.
15. MacDonald A, Dinah F, Mackenzie D, Wilson A. Performance feedback of hand hygiene, using alcohol gel as the skin decontaminant,

- reduces the number of inpatients newly affected by MRSA and antibiotic cost. *J Hosp Infect* 2004; 56: 56–63.
16. Hirschman H, Fux L, Podusel J et al. The influence of hand hygiene prior to the insertion of peripheral venous catheters on the frequency of complications. *J Hosp Infect* 2001; 49: 199–203.
 17. Gopal-Rao G, Jeanes A, Osman M et al. Marketing hand hygiene in hospitals: a case study. *J Hosp Infect* 2002; 50: 42–47.
 18. EN1040. *Chemical disinfectants and antiseptics. Basic bactericidal activity test methods and requirements. (phase 1)*. Brussels: CEN, 1996.
 19. EN1275. *Chemical disinfectants and antiseptics. Basic fungicidal activity test methods and requirements. (phase 1)*. Brussels: CEN, 1996.
 20. EN 1500. *Chemical disinfectants and antiseptics. Hygienic hand rub. Test method and requirements. (phase 2/step 2)*. Brussels: CEN, 1997.
 21. Herruzo-Cabrera R, Vizcaino-Alcaide MJ, Fernandez-Aceñero MJ. The influence of laboratory adaptation on test strains, such as *Pseudomonas aeruginosa*, in the evaluation of the antimicrobial efficacy of ortho-phthalaldehyde. *J Hosp Infect* 2004; 57: 217–222.
 22. Herruzo-Cabrera R, Vizcaino-Alcaide MJ, Fernandez-Aceñero MJ. Usefulness of an alcohol-based solution of *N*-duopropenide for the surgical antisepsis of the hands compared with handwashing with iodine-povidone and chlorhexidine: clinical essay. *J Surg Res* 2000; 94: 6–12.
 23. Pietsch H. Hand antiseptics: rubs vs. scrubs, alcoholic solutions vs. alcoholic gels. *J Hosp Infect* 2001; 48 (suppl A): 33–36.
 24. Kampf G, Ostermeyer C. Influence of applied volume on efficacy of 3 min surgical reference disinfection. Method prEN 12791. *Appl Environ Microbiol* 2004; 70: 7066–7069.
 25. Parienti JJ, Thibon P, Heller R et al. Hand-rubbing with an aqueous alcoholic solution vs traditional surgical hand-scrubbing and 30-day surgical site infection rates. *JAMA* 2002; 288: 722–727.
 26. Herruzo-Cabrera R, Garcia Torres V, Rey-Calero J, Vizcaino-Alcaide MJ. Evaluation of the penetration strength, bactericidal efficacy and spectrum of action of several antimicrobial creams against isolated microorganisms in a burn centre. *Burns* 1992; 18: 39–44.